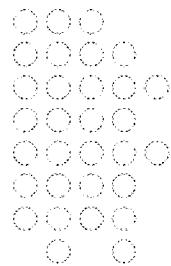
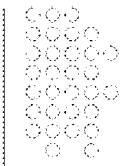


Autoclave Training

University of Massachusetts
Biological Safety Services



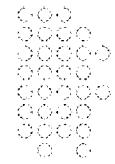
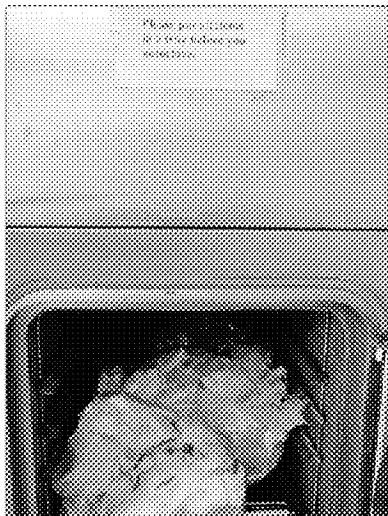


Learning Objectives

- What materials can be autoclaved?
- What PPE (personal protective equipment) is necessary for autoclave use?
- How does one properly load the autoclave?
- What steps assist in efficient decontamination of biohazardous waste?
- How does one verify autoclave performance for decontamination of biohazardous waste?

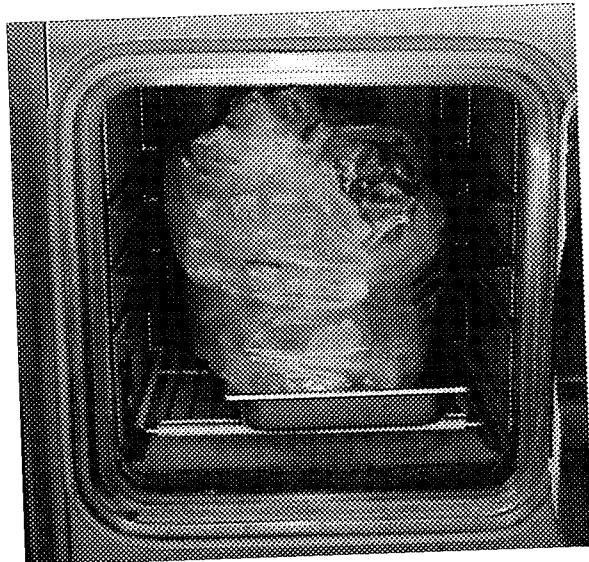
The learning objectives are the key points to take away from this training. You will learn operational techniques as well as important information to keep you safe while using this essential laboratory equipment.

What's Wrong With These Pictures?



View these pictures. Consider what the problems depicted here might be.
Both autoclaves have bags that are overfilled.
None of the bags are in a tray/bin.
The autoclave bag in the autoclave on the right has obstructed the drain.

What's Wrong With This Picture?



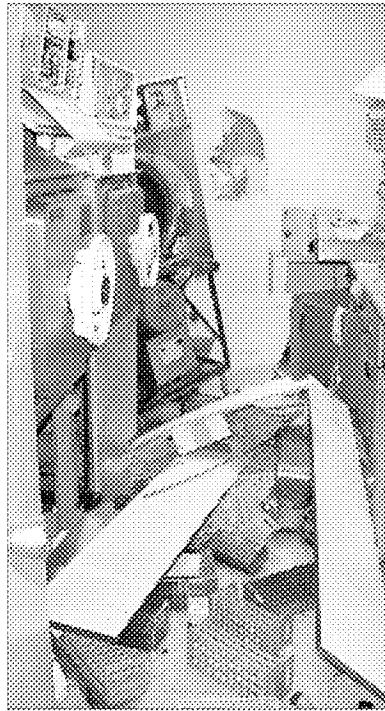
This is another problem to consider. The autoclave bag is overfilled and is too large for the tray. The bag comes very close to touching the sides, which is not desirable.

Importance of Proper Autoclave Use

- Tremendous pressure from steam in chamber provides explosive potential
- High temperatures and presence of extremely hot water creates potential for burns and scalding
- Inadequate decontamination allows for the potential of biological hazards and personnel and environmental contamination

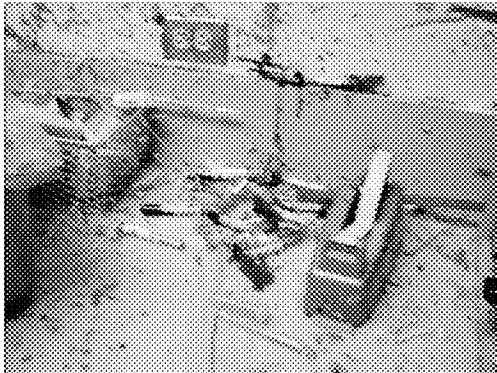


Autoclaves are pressure vessels that use steam to kill microbes. They are useful tools for decontamination, but they must be used appropriately. If autoclaves are used incorrectly, they can put you and the environment at risk.



Autoclave Explosion

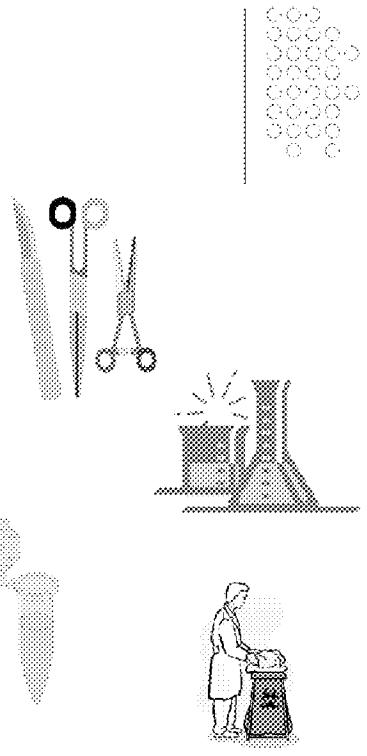
These pictures represent the results of an improperly closed autoclave door.



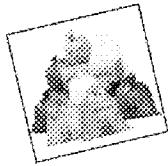
Improperly closed doors or damaged autoclaves can result in catastrophic events such as burns and even explosions. You need to know how to safely operate all equipment that you use.

What can be autoclaved

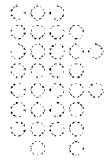
- Surgical Instruments
- Glassware
- Plastic tubes and pipette tips
- Solutions and water
- Animal food and bedding
- Waste



Autoclaves may be used to sterilize items for future use or to decontaminate waste before it is disposed of. Autoclaving for 30 minutes at 15 pounds of pressure per square inch @ 121C (250F), is the **minimum** run for steam sterilization.



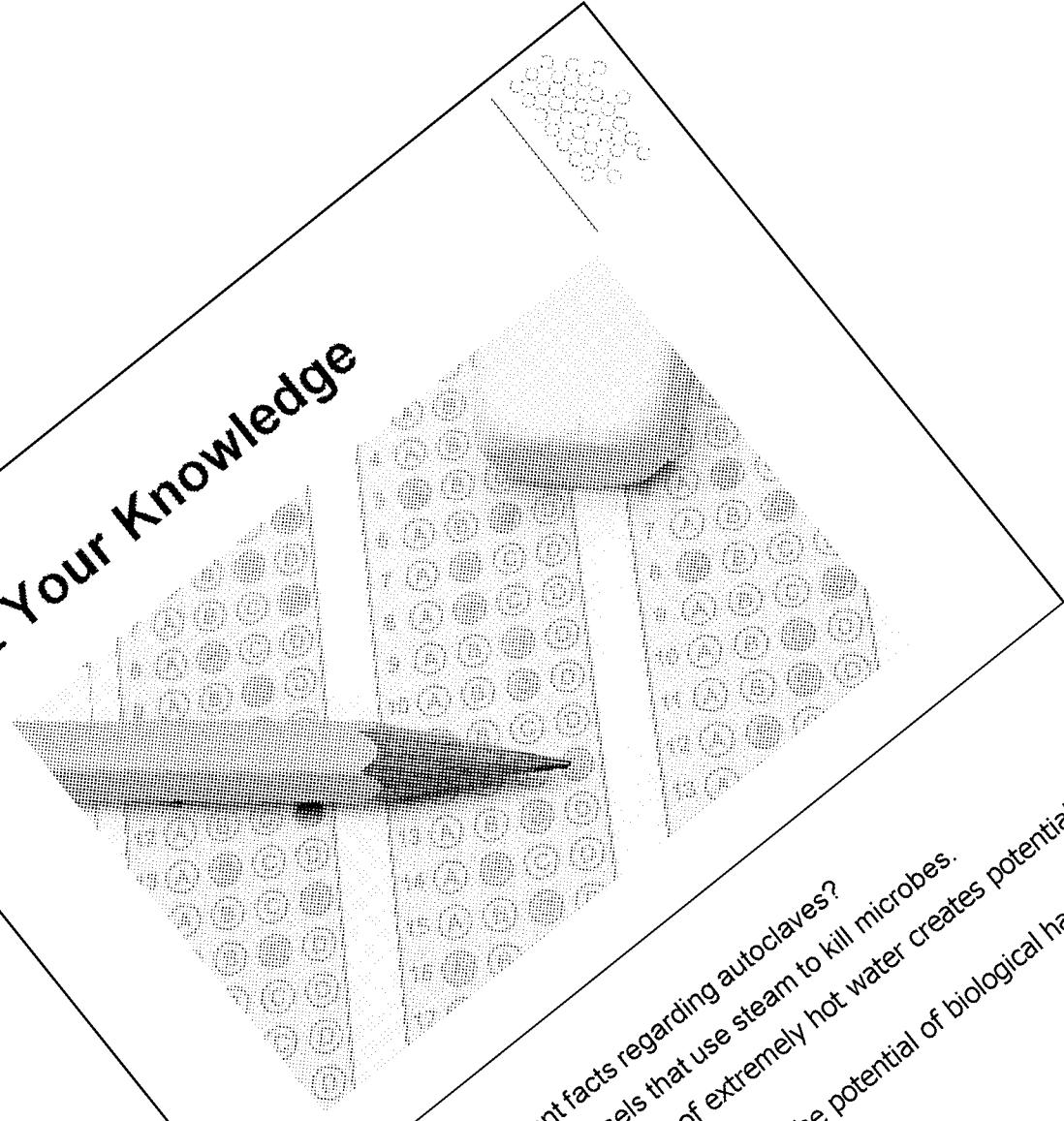
Types of Waste



Waste Type	Container	What You Need To Do
Biohazardous Waste (Serological Pipets, Pasteur Pipets, Pipet Tips)	Orange or red autoclave bag	Autoclave
Medical Waste Human tissue, Non-human primate tissue, Materials contaminated with blood or other human fluids	Red incineration bag in red bio-waste container	Contact EH&S for pick-up; Complete Hazardous waste request on website
Sharps- All needles, syringes, scalpels, blades, and all other sharp medical waste	Sharps container	Contact EH&S for pick-up; Complete Hazardous waste request on website
Animal Carcasses- no biological, chemical, or radiological hazard	Red incineration bag in fiberboard waste container. Freeze 48 hours.	Contact EH&S for pick-up; Complete Hazardous waste request on website
Used Biological Culture Media	Glass or plastic container	Disinfect with bleach or autoclave

This is a useful chart to refer to clarify waste streams.

Test Your Knowledge



What are some of the important facts regarding autoclaves?

- A. Autoclaves are pressure vessels that use steam to kill microbes.
- B. High temperatures and presence of extremely hot water creates potential for burns and scalding.
- C. Inadequate decontamination allows for the potential of biological hazards and personnel and environmental contamination.
- D. All of the above

What can be autoclaved?

- A. Surgical Instruments
- B. Solutions and water, animal food, animal bedding and waste
- C. Radioactive materials
- D. A and B only

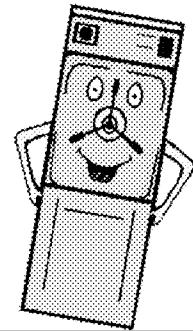
What color autoclave bags are deemed appropriate by the State of Massachusetts?

- A. Yellow
- B. Orange
- C. Red
- D. B and C

Principles of Autoclave Operation

- Steam penetrates objects in the autoclave
- Condensation creates negative pressure and draws in additional steam
- Moist heat kills microorganisms via coagulation of proteins
- Two types of autoclaves
 - Gravity Displacement
 - Vacuum Assisted

www.clipsahoy.com/clipart2/as3388.gif



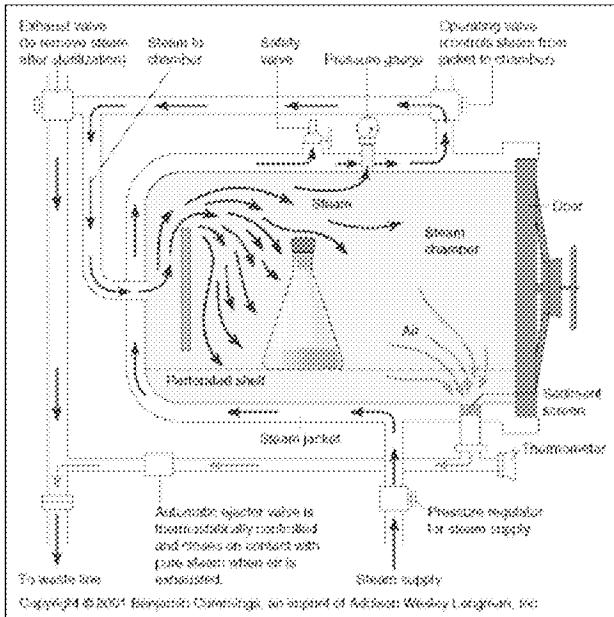
So let's apply some logic. You need liquid to generate steam. Some is supplied by the autoclave but you can add water to your load (inside bags) to help. If load is packed too tightly, the steam can not penetrate the center of the bags.

Principles of Autoclave Operation

- Thermal Death Time (TDT) is the time required to kill a known population of microorganisms in a specific suspension at a particular temperature
- Increasing the temperature decreases TDT
- Decreasing the temperature increases TDT
- Acidic or basic pH's decrease TDT
- Fats and oils slow penetration and increase TDT

If you manipulate the parameters of the autoclave and/or the waste, the outcome of sterilization is affected.

Gravity Displacement

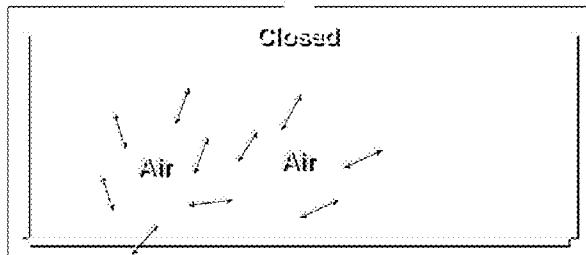
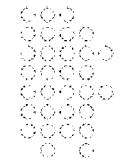


In a gravity displacement autoclave cold air escapes through the bottom of the chamber as steam displaces it from above. The valves can never be obstructed and the chamber must not be overfilled in order for this system to function efficiently.

There is a common thread to be repeated: do not overfill the autoclave so that steam can move freely.

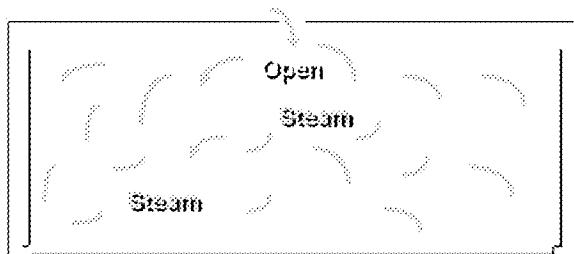
Drain valves must be kept clear. Bags must not be able to cover valves during the run. Keep bags in secondary containment.

Vacuum Assisted



A vacuum assisted autoclave removes cold air from the chamber prior to introduction of steam.

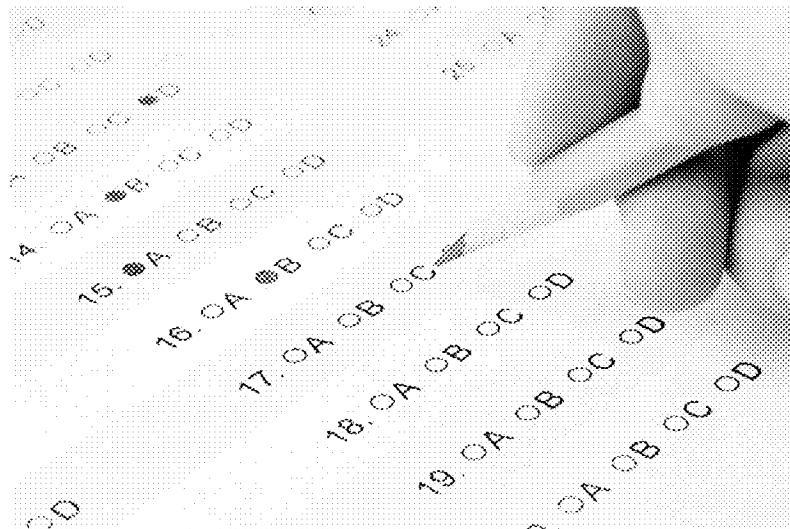
Open



Closed

Non-vacuum autoclaves are not very efficient at removing trapped pockets of air and, because microorganisms will not be destroyed unless they come into direct contact with steam, any air that remains trapped within the instrument or load will act as a barrier to the steam and prevent parts of the load from being sterilized.

Test Your Knowledge

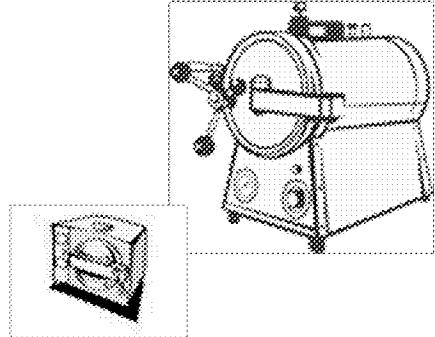


What can you do to make your autoclave more efficient?

- A. Place as much waste in the bags as possible
- B. Add water to the autoclave bags
- C.

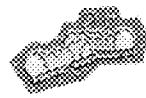
Autoclave Use Basics

- Personal Protective Equipment (PPE)
- Packaging
- Loading
- Operating
- User logs
- Maintenance logs
- Unloading
- Improper Autoclave Practices



www.fotosearch.com/thumb/ICL/ICL180/TEC_124C.jpg
www.affy.com/arts_crafts/clip_art?name=Science

An autoclave is a scientific instrument that needs to be operated with care and attention to detail. There are many facets to the use of the campus autoclaves and if you are required to use an autoclave, you are expected to be compliant with all aspects of its operation.



PPE for Autoclave Users

- Eye Protection
- Lab Coat, Buttoned
- Closed-toed Shoes
- Heat-resistant Gloves



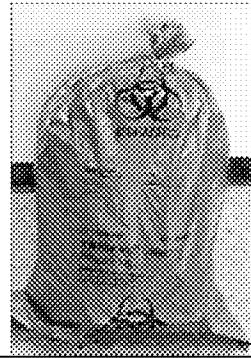
Autoclaves are pressure vessels that use steam at high temperatures. There are dangers when handling waste that goes into the autoclaves as well as the physical dangers regarding the unit itself.

Liquids pose a burn/explosion hazard as does the steam when the door is opened. It is important to be wearing the appropriate PPE when opening the door, moving liquids and handling any bio-waste itself.

A rubber apron or a heavy vinyl apron can be good protection when handling hot liquids. Do not wear cuffed pants. A solid shoe is better than an open weave sneaker in case of a splash. A face shield will protect your face when moving bottles.

Packaging for Autoclaving

- Be sure the material is safe to be autoclaved
 - No volatile or flammable chemicals, sharps, or radioactivity
- Utilize containers and autoclave bags appropriate for autoclaving
 - Red or orange bags only
 - States "Autoclave Bag"
 - Do not use other bags
- Do not overfill containers



Keep waste streams separate. Radioactive material should not be heated. Volatile chemicals should not be heated and placed under pressure.

If your waste bag does not state "Autoclave Bag" on it, do not use it in the autoclave!

Do not overload the autoclave.

Packaging for Autoclaving

- Carefully add water to autoclave bags
 - Add approximately 1 liter of water to 24" x 30" bag
 - Aids in reaching appropriate temperature quickly
 - Creates steam in the autoclave bag to replace cold air
- Prepare packaging to allow steam penetration
- Check that all containers including bags are vented
 - Loosely close autoclave bags
 - Vent lids on bottles containing solutions
- Place packaged material in a secondary container
 - Stainless steel tray or autoclavable polypropylene bin

To achieve the best decontamination, you must take the appropriate steps for packaging the waste.

Venting lids and bottles helps to prevent the rupture of bottles and/or caps blowing off.

Placing materials in a secondary container makes spill clean up easier if bags burst as well as avoiding drain blockage.

Sterilization Techniques

- **Fabrics and Hard Goods:**

- Use gravity cycle (or pre-vacuum if available)

- Limit size and density

- Arrange load for maximum exposure

- **Liquid**

- Use liquids cycle only

Fill liquid containers only half full.

Loosen caps or use vented closures.

Always put bags of biological waste into pans to catch spills.

Position biohazard bags on their sides, with the bag neck taped loosely.

Turn the container on its side when possible.

Select the container with the lowest sides and widest diameter possible for the autoclave.

Leave space between items to allow steam circulation.

Household dishpans melt in the autoclave. Use autoclavable polypropylene or stainless steel pans.

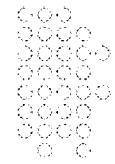
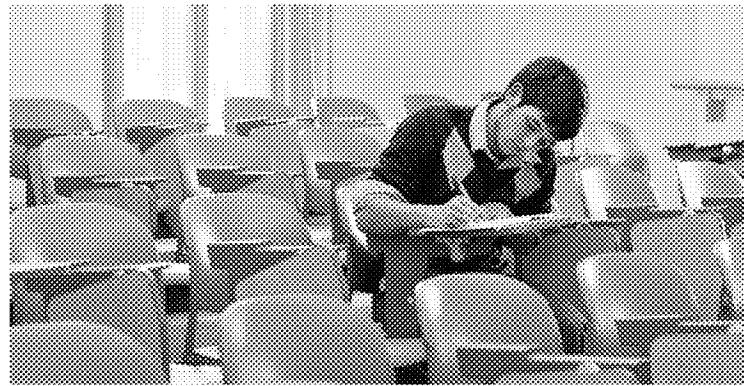
Use liquid cycle (slow exhaust) when autoclaving liquids, to prevent contents from boiling over.

Take into account the size of the articles to be autoclaved. A 2-liter flask containing 1 liter of liquid takes longer to sterilize than four 500 mL flasks each containing 250 mL of liquid.

Material with a high insulating capacity (animal bedding, high sided polypropylene containers) increases the time needed for the load to reach sterilizing temperatures.

Autoclave bags containing biological waste should be autoclaved for 50 minutes to assure decontamination.

Test Your Knowledge



When Mixing Loads

- Hard goods on lower shelves
- Fabrics on higher shelves
- Sterilize liquids separately!

Liquids require a separate autoclave setting and should not be autoclaved at the same time as other materials.

Burn Hazard When Sterilizing Liquids

- Loosen caps on containers of liquids before loading
- Use vented closures on bottles
- Use Type 1 borosilicate glass bottles (Pyrex)
- Place bottles of liquids in a pan and add water to the pan to avoid breaks
- Avoid sudden full opening of door at the end of cycle. Crack one inch, wait 20 minutes
- Bottles no more than 2/3 full

Check that the chamber pressure is zero.

Wear lab coat, eye protection, heat insulating gloves, and closed-toe shoes.

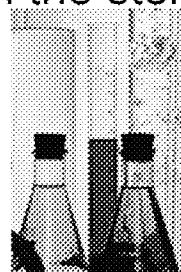
Stand behind door when opening it.

Slowly open door only a crack. Beware of rush of steam.

After the slow exhaust cycle, open autoclave door and allow liquids to cool for 20 minutes before removing.

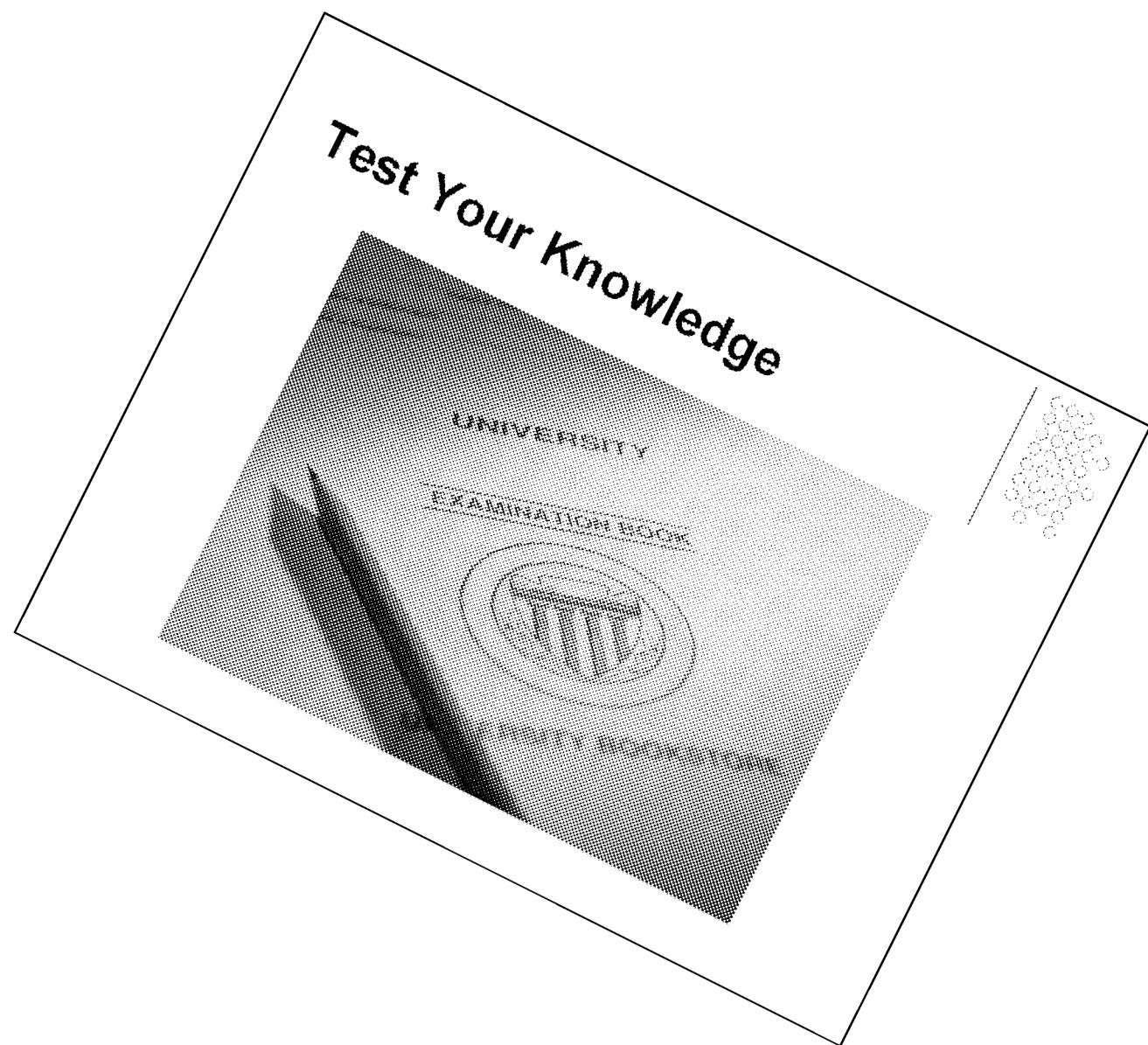
Burn Hazard When Sterilizing Liquids

- Use LIQUID cycle only.
- Do not jolt hot bottles. Do not move bottles if they are bubbling or boiling.
- Allow bottles to cool to touch before attempting to move them from the sterilizer shelf.



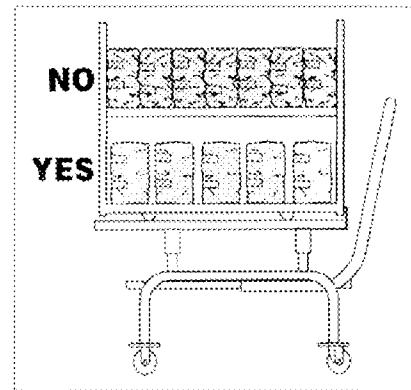
Burn Emergency:

If you are burned, you should seek medical treatment as soon as possible. Burns to the face, third-degree burns, or burns over large areas of the body should be treated as emergencies. Minor burns should be treated by using first aid procedures. These procedures would include immersing the burn in cool water immediately, removing clothing from the burn area, and keeping the injured area cool for at least five minutes (preferably longer). A drench shower may be used for this purpose. Any burns to the face or eye or any burns that blister should be seen by a physician. Regardless of the degree of severity, report the burn to your lab supervisor or Principal Investigator and EH&S as an occupational injury.



Loading the Autoclave

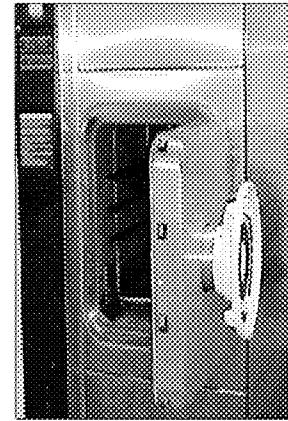
- Load material to allow efficient steam penetration
- Autoclave clean items and waste separately
- Do not allow material to be autoclaved to touch the sides or top of the chamber



Reminder: Do not overload the autoclave

Operating the Autoclave

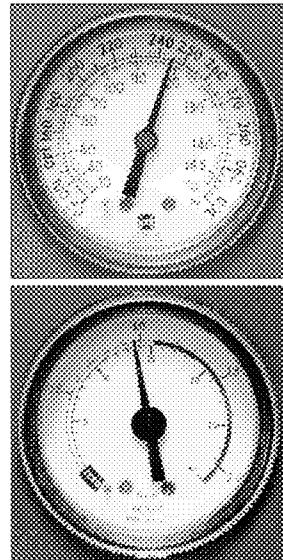
- Clean the drain strainer (trap)
- Be sure the autoclave is functioning properly before use
- Record information in Autoclave Log
- Close door properly and securely
- Choose the correct conditions for your material



Do not use the autoclave unless you have been trained on that unit in that department and feel comfortable with the use of the unit.

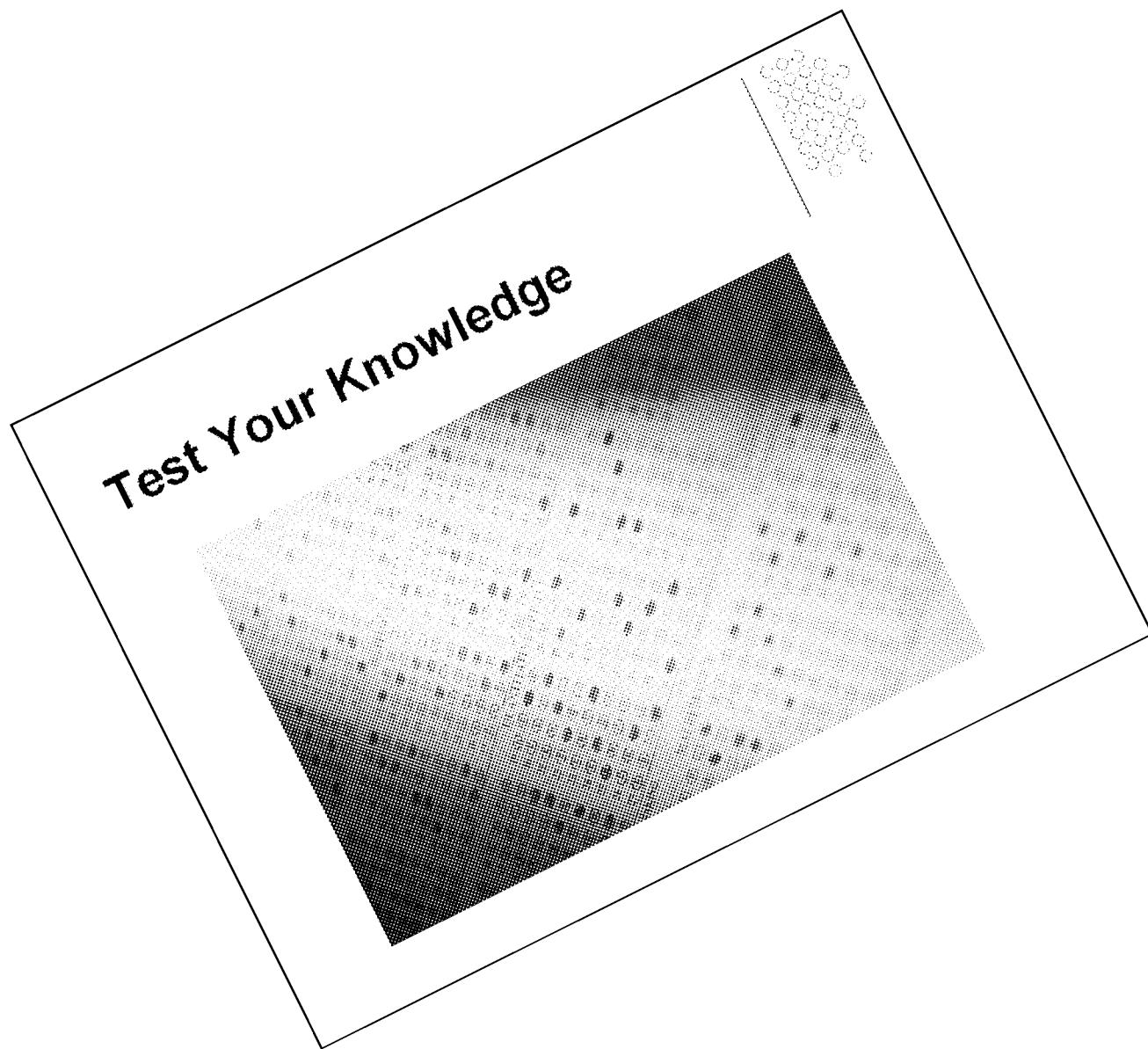
Operating the Autoclave

- For decontamination:
 - Temperatures between 121-124°C
 - Total processing time 60-120 minutes
 - Exposure time greater than 20 minutes
 - Pressure minimum 15 PSI
 - Select liquid cycle or slow exhaust cycle



Autoclaves “knock down” the bacterial count in waste by a log of 1.0×10^4 . What does this really mean? Autoclaves kill a large amount of the bacteria present in the load, but not all of it. It is rare the a load of waste is “sterile” after autoclaving.

Autoclaves take some time to “come up” to temperature and pressure, and this time must be subtracted from the total processing time.



Test Your Knowledge

Autoclave Waste Log

- Maintain User Logs for Three Years
- Complete User Log with Every Use
- Required by State of Massachusetts M.G.L.c. 111 §§ 3, 5 and 105 CMR 480.000
- See Autoclave Verification Program at <http://www.ehs.umass.edu/>

University of Massachusetts Amherst Biological Waste Record for On-Site Treatment
Department: Chemical Engineering Building: Lab II Room: 202 Manufacturer: MECO
Model #: MECO Serial #: _____ UMass Tag #: _____ Date Placed in Service: _____ Autoclave was: Used

Date	Quantity	Type	Treatment Method	Process Parameters	Printed Name	QC Results
			Heat			
			Heat			
			Heat			

The autoclave waste logs are mandatory and completing the log for each run of the autoclave is required.

Date: The date that the run is initiated

Quantity: This is the exact number (and size) of autoclave bags, bottles, flasks, racks, etc. that you have in the autoclave

Type: This column is to note what the purpose of autoclave run is. Waste (decontamination) or Sterilization (media, glassware, instruments, etc.)

Treatment Method: This is heat in an autoclave.

Process Parameters should vary with the type of load in the autoclave. Liquids require different cycles from dry waste, etc. The pH column remains blank for autoclaves.

Printed Name: Your name MUST be printed and it must be legible.

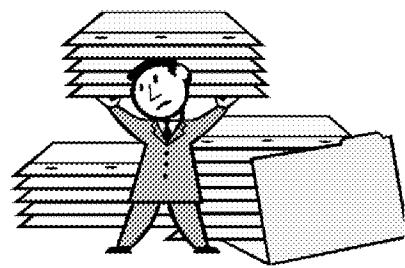
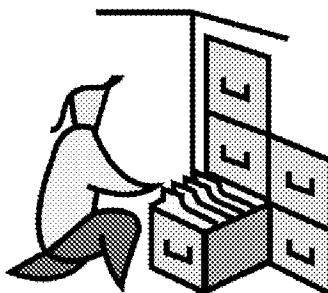
Signature: You MUST sign your name. You are verifying that you are meeting the minimum waste management requirements. The waste log is prepared in accordance with Massachusetts General Law c. 111 §§ 3, 5 and 105 CMR 480.000, minimum requirements for medical or biological waste.

QC Results: Note the type of quality control used and how it performed.

Example: Tape: Color Change ok; Bag: Color change ok; Bacterial Indicator: color change ok; Spore test: positive = + and Negative = -.

Autoclave Maintenance Log

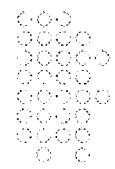
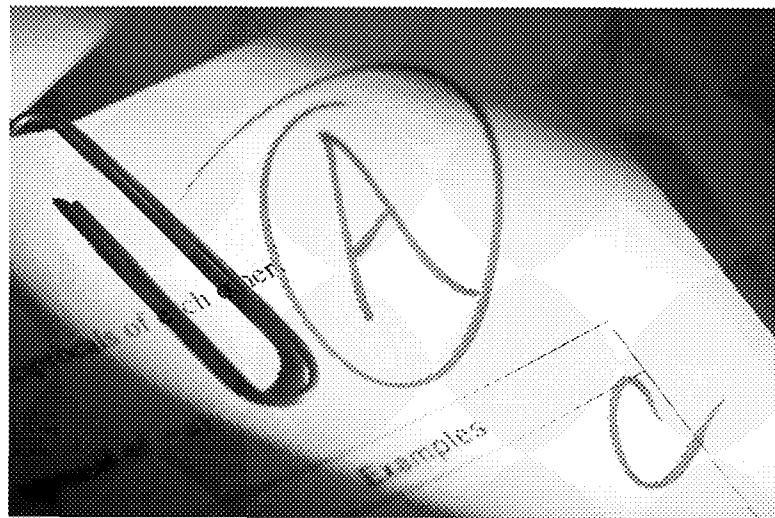
- Maintain Maintenance Logs for Five Years
- Complete Maintenance Log for Every Repair
- Maintain records of Yearly Calibration



Documentation shall be kept in the departmental office and entered into the Maintenance Log. The maintenance log consists of records of repairs, yearly calibrations, or any addition service.

All autoclaves are considered “pressure vessels” and must be calibrated annually to meet Massachusetts State regulations 105 CMR 480.000: Minimum Requirements for Medical or Biological Waste (State Sanitary Code Chapter VIII). Calibration consists of a preventative maintenance service which entails replacing valves and check valves that warrant it as well as door gaskets that may need replacing. Test cycles are run with chemical indicators to verify proper operation by the service representative(s).

Test Your Knowledge



Unloading the Autoclave

- Put on Personal Protective Equipment
- Allow the autoclave to completely finish cycle
 - Pressure gauge must read zero
- Verify cycle conditions were met
- Check chamber pressure before opening (0)
- Open door slightly to allow steam to escape
- Wait at least 10 minutes
- Allow contents to cool before removal
 - Be especially careful with fluids and plastic bins

PPE is necessary protection from heat, steam and explosive hazards.

Allowing the pressure to reach zero avoids breaching the door's safety mechanism and thus steer clear of burns, excessive steam and possible explosion.

Verifying cycle conditions is important. If time, temperature or pressure were not met, the load was not properly processed. Remedial action must be taken and then the load must be processed again.

Fluids are superheated after autoclaving. If they are jostled while they are still hot, they may begin to boil violently out of the container.

Plastic bins may flex allowing liquids to spill. Fully support bins when moving them.

Hazard Warnings

- Slip hazard: Wipe up spills on slippery floors
- Burn hazard: Remember that sterilizer racks and shelves will be HOT after cycle is run.
- Wear heat protective gloves, buttoned lab coat and safety glasses with face shield.



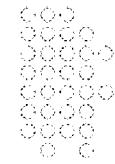
These safety reminders need to be followed each and every run. You are responsible for your safety and for those that share this work area.

Hazard Warnings

- Remove the load and let the glassware cool for 15 minutes before touching it with ungloved hands
- Allow autoclave and everything else to cool before cleaning or performing any maintenance
- Burn and shock hazard: Repairs and adjustments should be performed by authorized personnel only.
- Never clean autoclave with abrasives, wire brush, or steel wool.

Read the equipment manual for the autoclave that you use to become familiar with maintenance practices that are acceptable for you to perform vs. a professional.

Test Your Knowledge



Results of Improper Autoclave Use



Note that plastic bins must be heat resistant to withstand autoclaving. The bin on the right should have been placed on a rack as well. The bottom-most picture may have been a case where the bag was too big for the bin and tipped everything over on its side.

Autoclave Verification Program

- Program will ensure that all potentially hazardous biological waste is properly decontaminated prior to deposition in landfill
- Program requires testing of the ability of campus autoclaves to kill microorganisms
- There are over 70 autoclaves on campus which require this verification program

The purpose of the Autoclave Management Program for Biological Waste Decontamination is to comply with state regulations (105 CMR 480.000) and protect the public health, safety, welfare, and environment. Implementation of this program by all research laboratories within the University of Massachusetts at Amherst will ensure that all biological waste generated in the laboratories will be decontaminated and/or deactivated prior to disposal as solid waste.

Examples of biological waste are:

Human and animal cell lines

Tissue cultures

Organisms with recombinant DNA

Cultures and stocks of infectious agents and non-infectious microbial agents

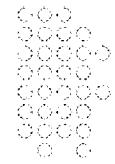
Bacteria, viruses, and fungi

Blood and blood products

Labware that has come into contact with the aforementioned waste streams (e.g. contaminated plastic pipettes, pipette tips, Petri dishes, centrifuge tubes, Eppendorf tubes, disposable gloves, wipes, etc.)

Autoclave Verification

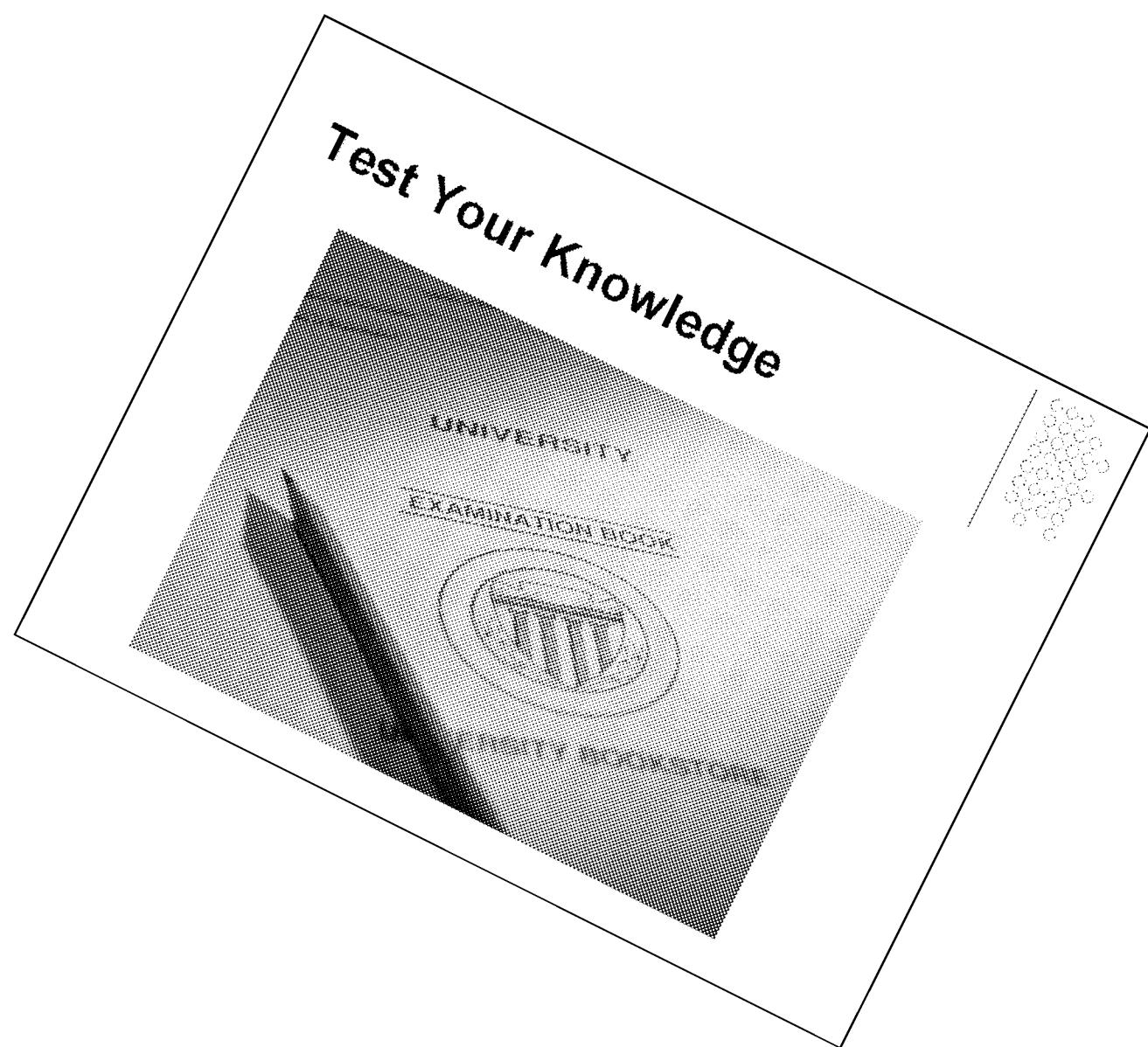
- Each Load of Biohazardous Waste
 - Autoclaves with automatic documentation
 - Review the printed report
 - If conditions were met, initial, date, and place printed report in nearby bin to save these
 - Document the run in the Autoclave Waste log
 - If conditions were not met, do not remove the load, repeat autoclave cycle.
 - If conditions are not met again, autoclave must be labeled out of order.
 - Contact responsible individual posted on autoclave.
 - Responsible individual contacts service company to schedule repairs.



Autoclave Verification

- Each Load of Biohazardous Waste
 - Autoclaves without automatic documentation
 - Autoclave tape must be incorporated in each load.
 - Based on color change the performance of the autoclave will be evaluated.

Performance of the autoclave must be verified for each run with indicator tape. The frequency of biological indicator (spore) testing will vary with the hazard levels of the waste (minimum = monthly). These policies are based on the requirements for processing waste as described in Biosafety in Microbiological and Biomedical Laboratories, fifth edition, HHS Publication No. (CDC) 93-8395.



Autoclave Verification

- Monthly Verification for Biological Waste Autoclaves
 - Biological Indicator Test
 - Checks all conditions of autoclave cycle, time, temperature, and pressure
 - Verifies ability of autoclave to kill a microorganism
 - Utilize the Prospore Biological Indicator
 - Record results in Autoclave Log



Spores are hermetically sealed in type I borosilicate glass ampoules. The ampoule is filled with a modified soybean casein digest broth containing a bromocresol purple acid indicator. Each ampoule also contains a population of *G. stearothermophilus* spores. Ampoule has a 48 hour incubation period. Growth is evident by either turbidity and/or a color change from a purple to orange toward yellow.

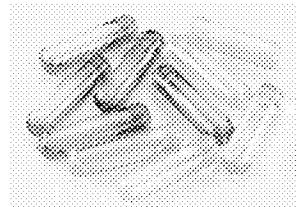
Biological Indicator Test Procedure

- Label package containing biological indicator vial with date
- Place the indicator vial package in the autoclave with the biohazard waste load
- Run the autoclave cycle as usual
- Using proper unloading procedures, remove the indicator package from the autoclave

To test autoclave performance with a biological indicator place a Prospore© ampoule in the most difficult location to sterilize, usually near the drain, between animal cages or suspended in a volume of liquid. Use as many ampoules as needed to get an accurate measurement of steam penetration throughout the load.

Biological Indicator Test Procedure

- Label the indicator vial with all information from package
- Check indicator vial label for color change from purple to yellow
- Place the biological indicator vial into the 56°C incubator



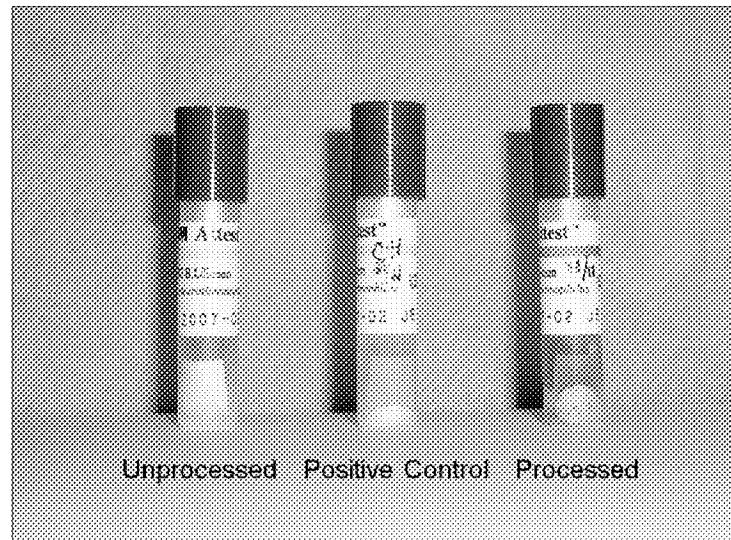
After the autoclave run place the processed ampoule in a vertical position in an incubator at 50-60 C for 48 hours. Mark a control ampoule from the same package and incubate it along with the autoclaved ampoule to ensure spore viability. Leave the ampoules at 50-60C for 48 hours.

Biological Indicator Test Procedure

- Place a non-autoclaved, labeled control tube in the incubator
- Examine the indicator tube at 8, 12, 24, and 48 hours for any color change
 - Yellow color change indicates bacterial growth
 - Compare to non-autoclaved control at each time point
- Record 48 hour test results in Results Log

These results are recorded on the “Autoclave Waste Log” under “QC Results”

Biological Indicator Test Results

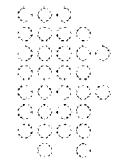
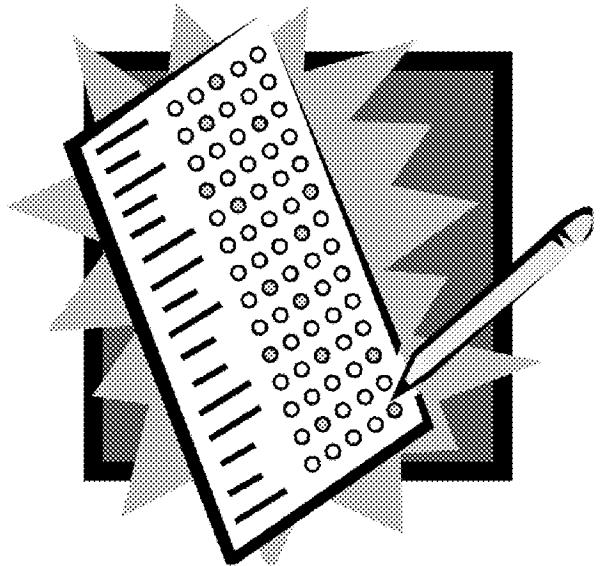


Control ampoule should exhibit a color change to or toward yellow. If the control ampoule does not exhibit growth via a color change the test is considered invalid because the spores are not considered to be viable.

Autoclaved ampoule will exhibit no growth and retain the original purple color if the autoclave is working properly meaning that both time and temperature were reached for decontamination. If the ampoule remains purple this indicates that the autoclave sterilization was successful. If the ampoule changes color towards a yellow or the liquid becomes turbid this indicates spore growth and failed autoclave sterilization.

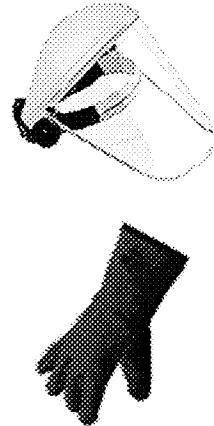
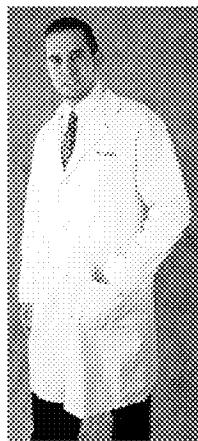
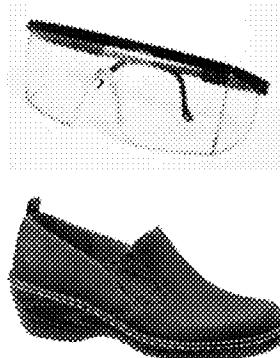
Test Failure If the control ampoule or the autoclaved Prospore[©] ampoule tests fails, the load must be re-autoclaved and retested with Prospores[©] again before disposal. The failure of a spore test may be the result of improper loading or incorrect parameter settings. If the autoclave spore test fails for a second time the autoclave needs to be serviced and use of the autoclave for decontamination suspended.

Test Your Knowledge



Learning Objectives Review

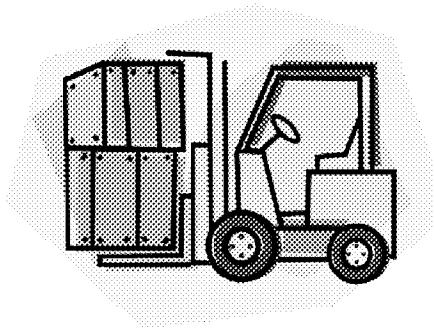
- What Personal Protective Equipment (PPE) is necessary for autoclave use?



- A. Glasses, closed shoes, lab coat, thermal gloves
- B. Closed shoes, face shield, lab coat, thermal gloves
- C. Face shield, glasses, closed shoes, lab coat, thermal gloves
- D. Any combination above

Learning Objectives Review

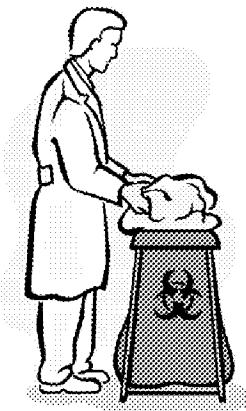
- How does one properly load the autoclave?



- A. Load material to allow efficient steam penetration
- B. Autoclave clean items and waste separately
- C. Do not allow material to be autoclaved to touch the sides or top of the chamber
- D. All of the above

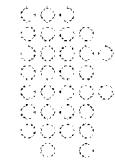
Learning Objectives Review

- What steps assist in efficient decontamination of biohazardous waste?



- A. Add approximately 1 liter of water to 24" x 30" loosely closed bag
- B. Place the bag directly on the bottom of the autoclave
- C. Place packaged material in an approved secondary container
- D. Answers A and C only

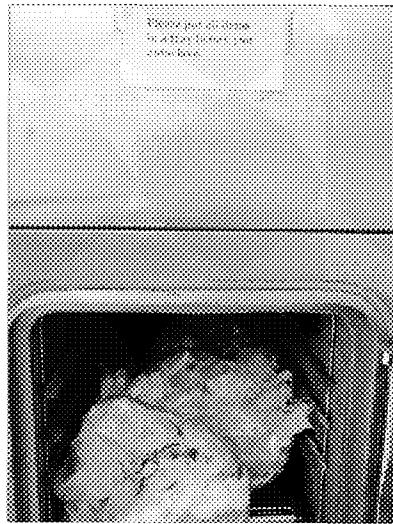
Learning Objectives Review



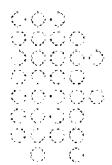
- How does one verify autoclave performance for decontamination of biohazardous waste?

- A color change is noted via autoclave tape or thermal indicator use
- You can make sure that there is no growth in the processed spore vial
- A and B
- Neither A and B

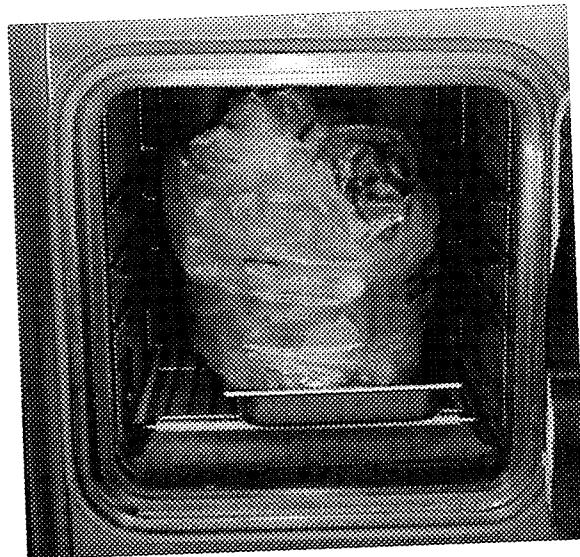
What's Wrong With These Pictures?



- A. Autoclave bags were not placed in a tray
- B. Autoclaves are overloaded
- C. One autoclave has a plugged drain
- D. All of the above



What's Wrong With This Picture?



- A. Autoclave bag is overfilled.
- B. Tray is too small.
- C. A and B are correct
- D. Neither are correct

Questions or Additional Information

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